Pharmaceutical Research, Vol. 13, No. 9, 1996

Research Article

Characterization of AUCs from Sparsely Sampled Populations in Toxicology Studies

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Received February 8, 1996; accepted June 17, 1996

Purpose. The objective of this work was to develop and validate blood sampling schemes for accurate AUC determination from a few samples (sparse sampling). This will enable AUC determination directly in toxicology studies, without the need to utilize a large number of animals. **Methods.** Sparse sampling schemes were developed using plasma concentration-time (Cp-t) data in rats from toxicokinetic (TK) studies with the antiepileptic felbamate (F) and the antihistamine loratadine (L); Cp-t data at 13–16 time-points (N = 4 or 5 rats/time-point) were available for F, L and its active circulating metabolite descarboethoxyloratadine (DCL). AUCs were determined using the full profile and from 5 investigator designated time-points termed "critical" time-points. Using the bootstrap (re-sampling) technique, 1000 AUCs were computed by sampling (N = 2 rats/point, with replacement) from the 4 or 5 rats at each "critical" point. The data were subsequently modeled using PCNONLIN, and the parameters (k_a, k_e, and V_d) were perturbed by different degrees to simulate pharmacokinetic (PK) changes that may occur during a toxicology study due to enzyme induction/inhibition, etc. Finally, Monte Carlo simulations were performed with random noise (10 to 40%) applied to Cp-t and/or PK parameters to examine its impact on AUCs from sparse sampling. **Results.** The 5 time-points with 2 rats/point accurately and precisely estimated the AUC for F, L and DCL; the deviation from the full profile was ~10%, with a precision (%CV) of ~15%. Further, altered kinetics and random noise had minimal impact on AUCs from sparse sampling. **Conclusions.** Sparse sampling can accurately estimate AUCs and can be implemented in rodent toxicology studies to significantly reduce the number of animals for TK evaluations. The same principle is applicable to sparse sampling designs in other species used in safety assessments.

KEY WORDS: toxicokinetics; sparse sampling; pharmacokinetics; toxicology.

INTRODUCTION

Blood samples are routinely obtained in drug safety studies to verify systemic exposure of animals to a test article and to obtain animal-to-human exposure multiples.[1] It is generally appropriate to base systemic exposure on the area under the plasma/serum concentration-time curve (AUC),[2,3] which is typically determined using intensive sampling schemes. In safety studies in large animals (e.g., dogs), the withdrawal of a sufficient number of blood samples for AUC determination is usually not difficult. In rodent studies, however, blood volume limitations restrict obtaining sufficient number of samples from individual animals. Therefore, separate (ancillary) toxicokinetic (A-TK) studies are often designed and conducted to obtain ~10–15 time-points with 4–5 animals/time-point, to fully characterize the AUC vs. dose relationship. Such studies involve a large number of animals and are resource intensive. Should the TK study be performed concurrent with the safety evaluation study, the number of rodents included for AUC estimation are usually more than those for the safety evaluations. Thus, there is clearly a need to streamline the TK programs so that they are conducted with minimal resources.

If AUCs can be determined from a few samples (sparse sampling), then they can be generated directly from animals in the safety studies, thereby precluding the need for the time-consuming A-TK studies. This would also enable direct correlation of plasma drug concentrations and toxicity findings within the same study, and lead to a more efficient preclinical drug development program.

The objective of this work was to develop and validate a method which will accurately estimate the AUC from a few samples in support of rodent toxicology studies. The goals of the sparse sampling approach were as follows: (1) AUCs must be both accurate and precise, (2) AUCs should be accurately captured from a sparse sampling scheme even when drug kinetics change, for example, over the duration of a toxicology study due to factors such as induction, saturation or accumulation, and (3) sparse sampling schemes should easily be implemented as part of a toxicology study. This paper describes how sparse sampling schemes were developed and validated

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0724-8741/96/0900-1283$09.50/0

using pharmacokinetic (PK) data from previous A-TK studies with the antiepileptic felbamate[4] and the antihistamine loratadine.[5] These two drugs were selected because of their range of PK and variability characteristics: (1) dose-related absorption and moderate variability (CV 20–30%) in Cp-t for felbamate, and linear pharmacokinetics and high variability (CV 40–70%) for loratadine, (2) major differences in the pharmacokinetic profiles of loratadine and its major circulating metabolite, descarboethoxyloratadine (DCL), and (3) quantitation of the metabolite's AUC from the same sparse sampling scheme used for the parent. Thus, for loratadine, sparse sampling schemes would be the most challenging to validate. Since new drug candidates usually show pharmacokinetic and variability characteristics within those for felbamate and loratadine, sparse sampling schemes developed and validated using these two drugs would be applicable to virtually all developmental drugs.

MATERIALS AND METHODS

Plasma Concentration-time Data

Plasma concentration-time (Cp-t) data in rats were from 28-day ancillary toxicokinetic (A-TK) studies with felbamate and loratadine conducted in support of the chronic safety studies. The test article was administered as a suspension (in 0.4% methylcellulose) once daily by gavage for 28 days at doses of 100, 300, and 1000 mg/kg for felbamate (males and females), and 10, 32, and 128 mg/kg for loratadine (males only). In the felbamate study, blood samples were obtained at 18 time-points at 0 (pre-dose) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 18, 24, 36, and 48 hr post-dose (N = 5 rats/sex/time-point/dose) on Days 1 and 28. In the loratadine study, blood samples were obtained on Day 28 at 13 time-points at 0 (pre-dose), and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 20, and 24 hr post-dose (N = 4 rats/time-point/dose). In both the felbamate and loratadine studies, each blood sample was obtained from an individual rat (i.e., by terminal sacrifice). Plasma samples were analyzed for felbamate by a HPLC method,[6] and for loratadine and DCL by GC with a nitrogen phosphorus detector.[7] The lower limits of quantitation (LOQ) were 0.25 μg felbamate/ml, and 0.2 ng/ml for both loratadine and DCL.

Pharmacokinetic Analyses

(a) Non-compartmental Analysis

The steady-state (Day 28) Cp-t data were analyzed by non-compartmental methods;[8] thus, 16 time-points were used for felbamate and 13 time-points were used for loratadine/DCL. The area under the plasma concentration-time curve over the dosing interval [AUC(24hr)] was calculated by the linear trapezoidal rule.[8]

(b) Compartmental Analysis

The steady-state Cp-t data at representative doses (100, 1000 mg/kg for felbamate and 32 mg/kg for loratadine/DCL) were analyzed using the non-linear least-squares regression program PCNONLIN.[9] The data were fitted to an open one-compartment PK model with first-order absorption and elimination (rate constants k_a and k_e, respectively). The estimated parameters were k_a, k_e, and the apparent volume of distribution (V_d). Plasma DCL concentrations were analyzed assuming first-order formation and elimination (rate constants k_f and k_{me}, respectively). A one compartment model was deemed most appropriate based on graphical inspection of the data and from Akaike's information criterion.[10] Goodness-of-fit was determined using (1) standard errors of the estimated parameters, (2) minimization of the sum of squared deviations, and (3) graphical inspection of observed vs. predicted Cp-t.

Random Sampling and Minimization of Data-Points

(a) Selection of "Critical" Time-points

The original profiles at all doses with the 13 or 16 time-points were inspected and 3, 4 or 5 time-points at which inflections occurred in the profiles were chosen. Then, AUCs were computed from the 3, 4, or 5 time-points, and were compared against AUCs from the full profiles, to determine which of the schemes with the reduced time-points (i.e., 3, 4, or 5) yielded AUCs with the least amount of bias. "Critical" points were then defined as those that yielded the most accurate AUCs. For felbamate, the "critical" time-points were 0, 2, 6, 10, and 14 hr, and for loratadine/DCL the "critical" time-points were 0, 1, 4, 6, and 12 hr. In the case of loratadine, the "critical" time-points were those which yielded accurate AUCs for both the parent and the active metabolite. For all three compounds, the Cp at 0 hr was also used at 24 hr for the computation of AUCs.

(b) Selection of Cp-t Using the Bootstrap (re-sampling) Technique

At each "critical" time-point, the Cp-t from individual animals (5 and 4 for felbamate and loratadine/DCL, respectively) were assigned a random number. Then, a single Cp-t was randomly chosen from the possible values (i.e., 5 and 4 for felbamate and loratadine/DCL, respectively), and its concentration value was recorded. The Cp-t was replaced, the samples randomized again, and a second Cp-t was (randomly) chosen and its value was also recorded. Thus, at each "critical" time-point, two Cp-t values were randomly selected; they were averaged and the AUC was computed from the 5 "critical" time-points. This process was repeated 1000 times, with fresh randomization prior to each "sampling", to obtain 1000 AUCs from which the mean and coefficient of variation (%CV) were determined. All computations were performed using SAS,[11] version 6.08, on an IBM mainframe computer Model 9121.

Simulation of Time or Dose-variant Alterations in Pharmacokinetics

Using the estimated PK parameters (k_a, k_e, and V_d) from compartmental modeling, Cp-t data were simulated at time-points at which samples had been obtained in the original TK studies (i.e., 16 for felbamate and 13 for loratadine/DCL). Subsequently, the parameters were perturbed by varying degrees to simulate time and/or dose-dependent changes that may occur over the course of a toxicology study. The extent of the perturbations, expressed as a multiple of the original (fitted) parameter

value were 0.1, 0.5, 5 and 10 for k_a; 0.5 and 2 for k_e; and 0.7 and 1.3-fold (i.e., ±30%) for V_d. In addition, k_a and k_e (k_f and k_{me} for DCL) were altered together in certain fixed combinations as follows: $10xk_a$ & $0.5xk_e$; $10xk_a$ & $2xk_e$; $0.1xk_a$ & $0.5xk_e$; $0.1xk_a$ & $2xk_e$, to reflect simultaneous changes in both the absorption and elimination rate constants (formation and elimination for DCL). In some simulations, certain combination of parameters (e.g., $0.1xk_a$ & $0.5xk_e$) resulted in 'flip-flop' kinetic phenomena. Since accumulation kinetics are governed by, among other factors, the input and elimination rates,[12] the new steady-state concentrations that may have resulted from altered PK were determined by the superposition principle.[13] For each set of perturbations, theoretical Cp-t values were generated at all time-points and also at the "critical" points. The AUCs from the two sets of data were compared to determine the degree of bias as a result of altered pharmacokinetics.

Monte Carlo Simulations

Using the 'RANNOR' function in SAS,[11] random noise was applied to plasma concentrations and to the pharmacokinetic parameters (k_a and k_e), either separately or together. The noise was controlled at 0 (i.e., no noise), and at 10, 20, 30, and 40% of the theoretical value. Then, applying a one-compartment model to the parameters with the added noise, new Cp-t data were simulated at all time-points and at the "critical" points; in some experiments noise was added to Cp-t also. At each noise level, this process was repeated to obtain 1000 AUCs from which the mean and %CV were computed. The estimates were compared for the full and sparse profiles to determine the impact of random noise on mean AUCs and their precision from sparse sampling.

Estimation of Accuracy and Precision of AUCs from Sparse Sampling

The accuracy (extent of bias) and precision (reproducibility) of AUCs from sparse sampling were determined using the following equations. In equation (2), the standard deviations (SD) were obtained from the 1000 AUC estimates.

$$\% \text{ bias} = \{(AUC_{sparse} - AUC_{full})/AUC_{full}\} \times 100 \quad (1)$$

$$\text{Precision (\% CV)} = (\text{SD/mean}) \times 100 \quad (2)$$

RESULTS

Representative mean plasma concentrations of felbamate, loratadine and DCL and the data from individual animals are shown in Figures 1 and 2. The AUCs from intensive vs. sparse sampling for felbamate are shown in Table 1, and those for loratadine and DCL are shown in Table 2. For felbamate, two representative profiles from sparse sampling are compared against the full profile in Figure 3.

For felbamate, at the low, mid, and high doses, AUC_{sparse} deviated from AUC_{full} by only −6, 0.4, and 6%, respectively (Table 1). For loratadine, at doses of 10, 32, and 128 mg/kg, AUC_{sparse} deviated from AUC_{full} by only 11, 12, and 4%, respectively, for the parent, and by only 11, −6, and 5% for DCL (Table 2). The %CVs based on the 1000 sampling experiments were ~10% for felbamate and ~15% for loratadine/DCL demonstrating high precision. Thus, for felbamate, loratadine, and DCL, the sparse sampling scheme accurately and precisely estimated the AUCs across all doses.

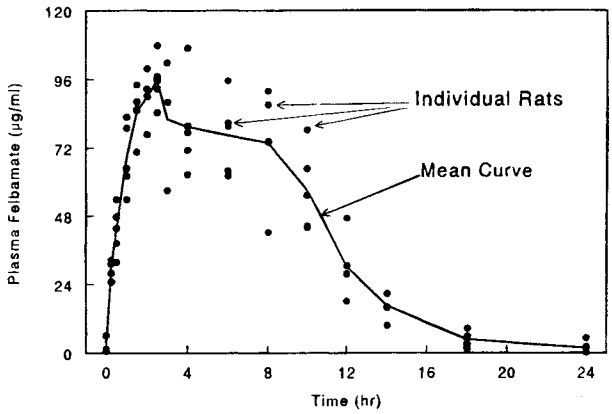
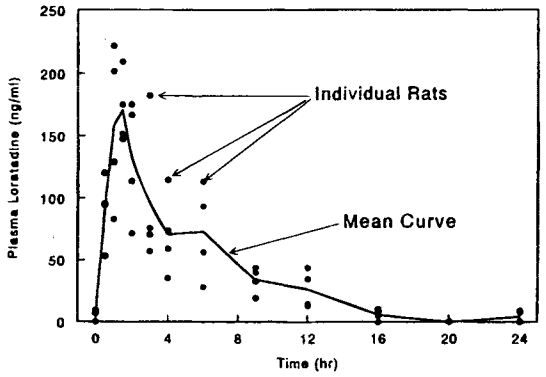


Fig. 1. Plasma Felbamate Concentrations: Mean Profile and Data in Individual Rats at a Representative Dose (300 mg/kg).



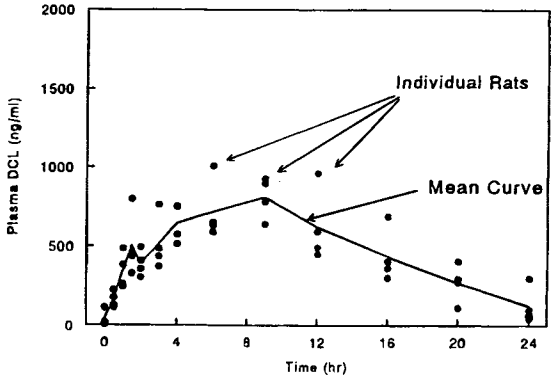


Fig. 2. Plasma Loratadine and DCL Concentrations: Mean Profiles and Data in Individual Rats at a Representative Dose (32 mg/kg).

Artificially perturbing the pharmacokinetics did not significantly affect the AUCs from sparse sampling (Tables 3 and 4). For felbamate, when k_a alone was perturbed 0.1, 0.5, 5, and 10-fold, AUC_{sparse} deviated from AUC_{full} by only −1, −5, −13, and −14%, respectively, for the 100 mg/kg dose, and −0.4, −1, −6, and −10%, respectively, for the 1000 mg/kg dose (Table 3 and Figure 4). When k_e alone was per-

Table 1. Area Under the Plasma Felbamate Concentration-Time Curve from Intensive (Full) and Sparse Sampling Schemes in Rats

Gavage Dose (mg/kg)	AUCs from Intensive and Sparse Sampling Schemes (μg·hr/ml) [Mean (%CV)]		
	16 Time-points (5 rats/point)[a]	5 Time-points (2 rats/point) (1000 replications)[b]	% Difference (AUC_{sparse} vs. AUC_{full})
100	372	348 (7)	−6
300	930	934 (6)	+0.4
1000	2014	2134 (8)	+6

[a] Steady-state (Day 28) data in male rats with 5 rats/Cp-t from an ancillary TK study.
[b] Sampling with replacement; 1000 curves were generated. Time-points were 0, 2, 6, 10, 14 hr and 24 hr (same as 0 hr concentration).

turbed 0.5 and 2-fold, the deviations were −4 and −12%, respectively, for the 100 mg/kg dose, and −1 and −3%, respectively, for the 1000 mg/kg dose. Perturbation of V_d positively and negatively by 30% (i.e., 0.7 and 1.3-fold) resulted in deviations of +8 and −8% for the 100 mg/kg dose and +4 and −4% for the 1000 mg/kg dose. Perturbing k_a and k_e together by varying degrees also resulted in accurate estimates of AUC_{sparse}. In the case of loratadine/DCL, as with felbamate, perturbing k_a and k_e (k_f and k_{me} for DCL) resulted in AUC_{sparse} values with deviations of ≤2% from AUC_{full} (Table 4 and Figure 5).

Table 2. Area Under the Plasma Loratadine Concentration-Time Curve from Intensive (Full) and Sparse Sampling Schemes in Rats

Parent (Loratadine)

Gavage Dose (mg/kg)	AUCs from Intensive and Sparse Sampling Schemes (ng·hr/ml) [Mean (%CV)]		
	13 Time-points (4 rats/point)[a]	5 Time-points (2 rats/point) (1000 replications)[b]	% Difference (AUC_{sparse} vs. AUC_{full})
10	257	284 (15)	+11
32	939	1050 (15)	+12
128	3387	3539 (8)	+4

Metabolite (DCL)

Gavage Dose (mg/kg)	AUCs from Intensive and Sparse Sampling Schemes (ng·hr/ml) [Mean (%CV)]		
	13 Time-points (4 rats/point)[a]	5 Time-points (2 rats/point) (1000 replications)[b]	% Difference (AUC_{sparse} vs. AUC_{full})
10	1829	2034 (11)	+11
32	11811	11074 (13)	−6
128	41131	43165 (6)	+5

[a] Steady-state (Day 28) data in male rats with 4 rats/Cp-t from an ancillary TK study.
[b] Sampling with replacement; 1000 curves were generated. Time-points were 0, 1, 4, 6, 12 hr, and 24 hr (same as 0 hr concentration).

Table 3. Effect of Artificially Perturbed Pharmacokinetics on Felbamate AUCs from Sparse Sampling

Parameter Perturbed	Perturbation Factor	% Difference (AUC_{sparse} vs. AUC_{full})[a]	
		100 mg/kg	1000 mg/kg
No Perturbation	—	−8	−4
Absorption rate constant (k_a)	0.1	−1	−0.4
	0.5	−5	−1
	5	−13	−6
	10	−14	−10
Elimination rate constant (k_e)	0.5	−4	−1
	2	−12	−3
Volume of distribution (V_d)	−30%	−8	−4
	+30%	+8	+4
Absorption & elimination rate constants	$10xk_a$, $0.5xk_e$	−7	−5
	$10xk_a$, $2xk_e$	−24	−17
	$0.1xk_a$, $0.5xk_e$	−1	−0.4
	$0.1xk_a$, $2xk_e$	−4	−1

[a] Intensive (full) sampling: 16 time-points. Sparse sampling: 5 critical time-points.

Monte Carlo simulations (Table 5) showed that when noise was introduced either in Cp-t ("assay" noise), or PK parameters (k_a, k_e), separately and together, the sparse sampling scheme still accurately estimated the true AUC. When assay and parameter noise were as high as 40%, the deviation of AUCs (sparse vs. full) were <10%. The noise, however, resulted in larger CVs. For felbamate, when only assay noise was introduced at the 10% level, the CVs (1000 simulations) were 1% and 2% for AUC_{full} and AUC_{sparse}, respectively; these increased to 5% and 9% when the noise was increased to 40%. When only parameter noise was present (at the 30% level), the CVs were 14% and 13% for the intensive and sparse schemes, respectively; when both assay and parameter noise were 40%, the CVs were 18% and 20%, respectively, for the intensive and

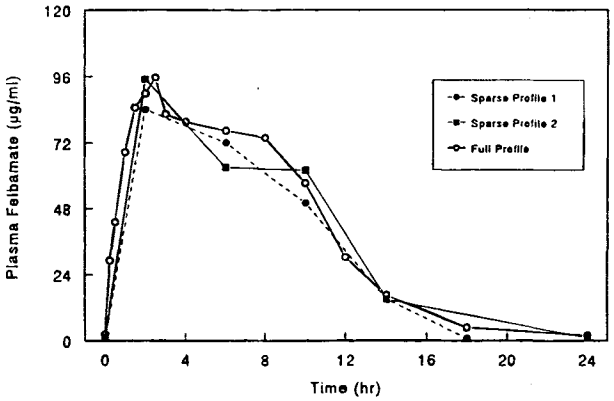


Fig. 3. A Comparison of Plasma Felbamate Concentrations from the Full Profile (all time-points) and Two Representative Profiles from Sparse Sampling (5 time-points with 2 rats/point) at a Dose of 300 mg/kg.

sparse sampling schemes. Similar results were obtained for loratadine.

DISCUSSION

The results of these analyses demonstrate that AUCs can accurately and precisely be determined from sparse sampling. The selection of drugs used in these analyses was based on: (1) variability in plasma concentrations at individual time-points, (2) pharmacokinetic characteristics and shapes of the Cp-t profiles, and (3) quantitation by sparse sampling of an active metabolite in addition to the parent compound. At individual time-points, felbamate showed moderate variability (CV 20–30%) and less than proportional increases in AUC with dose (Table 1), whereas loratadine was highly variable (CV 40–70%) due to its extensive first-pass metabolism; the metabolite DCL's variability (%CV) was ~30–40%. Both drugs were rapidly absorbed (Figures 1 and 2), but DCL, as expected of a metabolite, showed gradual increases in plasma concentrations. Thus, the major differences in the PK profiles of loratadine and DCL (Figure 2) offered the challenge of quantitating the AUCs of both moieties using the same sparse sampling scheme.

The extensive original data enabled rigorous validation of the reliability of AUC estimation from sparse sampling. The validation included an evaluation of the impact of altered kinetics, and of random noise, on the stability of AUCs from sparse sampling. It was found that at least 5 time-points were needed to accurately estimate the AUCs; schemes with 3 or 4 points, which were reductions of the basic 5 time-point scheme, generally overestimated the AUC (Table 6). For all three compounds, the Cp at 0 hr was also used at 24 hr for the computation of AUCs since the evaluations were made at steady-state; this approach is also appropriate for single-dose data with rapidly eliminated drugs.

Table 4. Effect of Artificially Perturbed Pharmacokinetics on Loratadine AUCs from Sparse Sampling (Dose: 32 mg/kg)

Parent (Loratadine)

Parameter Perturbed	Perturbation Factor	% Difference (AUC_{sparse} vs. AUC_{full})[a]
No Perturbation	—	+6
Absorption & elimination rate constants	$10xk_a$, $0.5xk_e$	+0.9
	$10xk_a$, $2xk_e$	−0.7
	$0.1xk_a$, $0.5xk_e$	+1
	$0.1xk_a$, $2xk_e$	0

Metabolite (DCL)

Parameter Perturbed	Perturbation Factor	% Difference (AUC_{sparse} vs. AUC_{full})[a]
No Perturbation	—	+0.9
Formation & elimination rate constants	$10xk_f$, $0.5xk_{me}$	+0.2
	$10xk_f$, $2xk_{me}$	+2
	$0.1xk_f$, $0.5xk_{me}$	−0.4
	$0.1xk_f$, $2xk_{me}$	−0.5

[a] Intensive (full) sampling: 13 time-points. Sparse sampling: 5 critical time-points.

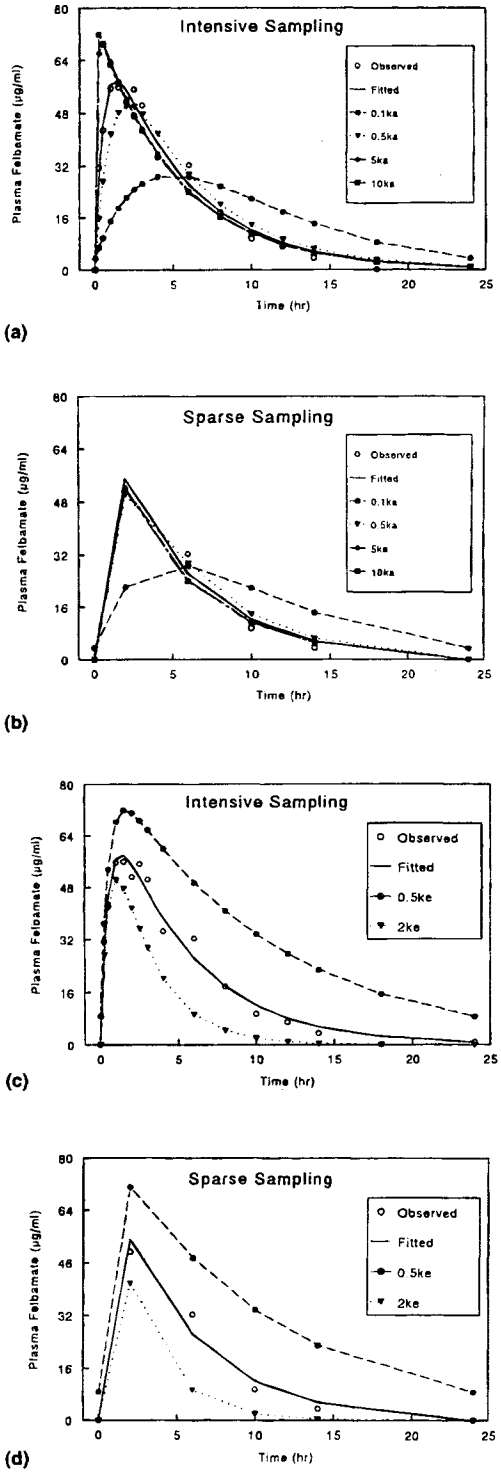


Fig. 4. Impact of Artificially Perturbed Pharmacokinetics on Plasma Felbamate Concentration-time Profiles from Sparse Sampling in Rats. a, b, changes in k_a; c, d, changes in k_e.

The impact of the reduction of the number of animals/time-point on AUCs was evaluated using the re-sampling (bootstrap) technique.[14] The bootstrap method uses the data at hand to compute a statistic by re-sampling the data with replacement. As an example, to compute the bootstrap estimate of the sample mean from a data set with "n" observations, the following algorithm is

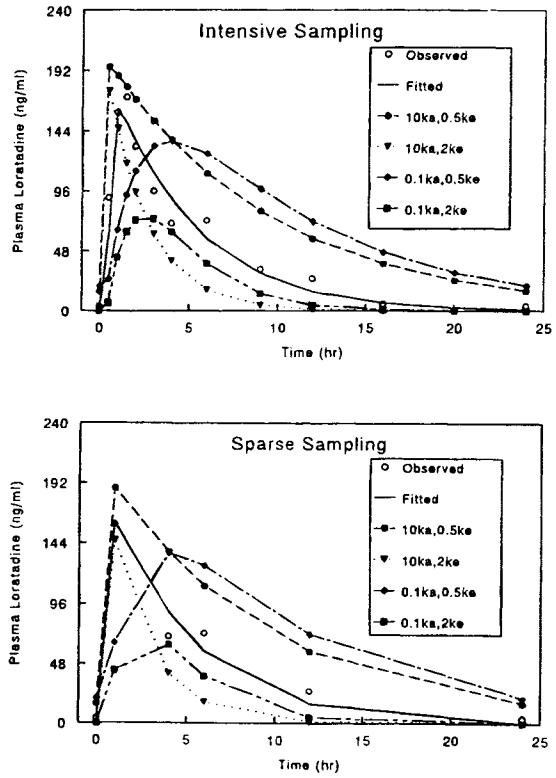


Fig. 5. Impact of Artificially Perturbed Pharmacokinetics on Plasma Loratadine Concentration-time Profiles from Sparse Sampling in Rats (Changes in both k_a and k_e).

used: (1) random selection from the data "n" observations with replacement, (2) computation of the sample mean, (3) repetition of steps 1 and 2 a large number of times (e.g., 1000), and (4) computation of the mean of the re-sampled means to obtain the bootstrap estimate. The bootstrap method is used to obtain better estimates than the usual asymptotic normal theory. For the problem of computing variance estimates for the AUC, the bootstrap method has been shown by Mager and Göller to perform satisfactorily against the usual asymptotic normal methods.[15]

The results of the re-sampling experiments showed that, for both felbamate and loratadine, the CVs were ~10% and ~15%, respectively, demonstrating that AUC_{sparse} was estimated with very high precision. The somewhat higher CVs for loratadine (Table 2) are expected because of greater variability in Cp-t associated with its extensive first-pass metabolism. It was noted that the CVs for the AUCs were significantly smaller than those at the individual time-points. This was an expected finding because: (1) AUC being an integral parameter minimized the effect of variability at individual Cp-t, and (2) the two animals at each time-point imparted stability.

A significant concern in sparse sampling designs in toxicology studies are the potential changes in pharmacokinetics with dose or over time due to, for example, changes in animal physiology, impaired metabolism due to toxicity, enzyme induction/inhibition, accumulation, etc. Changes in drug kinetics may translate to changes in the shapes of the concentration-time profiles, and a sampling scheme chosen based on a short term (or single dose) study may not accurately capture the AUC in a long term study. The impact of pharmacokinetic changes

Table 5. Effect of Random Noise on AUC Accuracy and Precision for Felbamate and Loratadine in Rats

Felbamate (100 mg/kg)

Source of Variability (%) PK Parameters (k_a, k_e)	Assay[a]	AUC (24hr) [mean (%CV)] N = 1000 simulations (μg.hr/ml) Full Profile[b]	Sparse Sampling[d]	% Difference (AUC_{sparse} vs. AUC_{full})
0	10	390 (1)	360 (2)	−8
0	20	390 (2)	359 (5)	−8
0	30	390 (4)	361 (7)	−7
0	40	390 (5)	360 (9)	−8
30	0	400 (14)	369 (13)	−8
30	20	400 (14)	370 (14)	−8
30	40	398 (14)	368 (16)	−8
40	40	406 (18)	374 (20)	−8

Loratadine (32 mg/kg)

Source of Variability (%) PK Parameters (k_a, k_e)	Assay[a]	AUC (24hr) [mean (%CV)] N = 1000 simulations (ng.hr/ml) Full Profile[c]	Sparse Sampling[d]	% Difference (AUC_{sparse} vs AUC_{full})
0	10	875 (1)	931 (2)	6
0	20	876 (3)	931 (4)	6
0	30	874 (4)	928 (6)	6
0	40	877 (6)	932 (9)	6
40	0	894 (17)	945 (15)	6
40	20	897 (17)	945 (16)	5
40	40	901 (18)	951 (18)	6

[a] Constant CV over concentration range.
[b] 16 Cp-t-time-points.
[c] 13 Cp-t time-points.
[d] 5 critical time-points.

on AUC estimates from sparse sampling was evaluated by artificially altering the pharmacokinetics (Figures 4 and 5). The extent of change was dependent on the degree by which the parameters were perturbed. For example, for felbamate, increasing k_a 10-fold resulted in a curve with a very rapid absorptive phase, and decreasing it to 1/10th the original value resulted in a curve with gradually increasing concentrations, as a result of 'flip-flop' kinetics (Figure 4). Perturbing both k_a and k_e resulted in an intermediate profile. It was found that sparse sampling accurately captured the AUC even when the parameters were perturbed by extreme degrees (e.g., 100-fold for the extreme limits of k_a) (Tables 3 and 4). For example, for felbamate, the deviations were typically ~10%; in an extreme perturbation (k_a and k_e increased together 10 and 2-fold, respectively) the deviations were only ~20%. Thus, the results showed that

Table 6. Minimization of the Number of Time-points Required for AUC Determination

Felbamate

Gavage Dose (mg/kg)	% Difference (AUC_{sparse} vs. AUC_{full})[a] 3 Points (0,2,6 hr)	4 Points (0,2,6,10 hr)	5 Points (0,2,6,10,14 hr)
100	+37	−0.5	−6
300	+22	+19	+0.5
1000	+14	+15	+5

Loratadine and DCL

Gavage Dose (mg/kg)	% Difference (AUC_{sparse} vs. AUC_{full})[a] Loratadine 3 Points (0,1,12 hr)	Loratadine 4 Points (0,1,4,12 hr)	Loratadine 5 Points (0,1,4,6,12 hr)	Descarboethoxyloratadine 3 Points (0,1,12 hr)	Descarboethoxyloratadine 4 Points (0,1,4,12 hr)	Descarboethoxyloratadine 5 Points (0,1,4,6,12 hr)
10	+110	+9	+11	−3	+24	+11
32	+37	+6	+12	−20	−9	−7
128	+32	−2	+4	+12	+8	+5

[a] 24 hr same as 0 hr for all sampling schemes.

a sparse sampling scheme chosen based on previous information regarding Cp-t profiles would accurately capture the AUC even if the pharmacokinetics changed significantly with time.

The results of the Monte Carlo simulations lend further support to the stability of AUCs from sparse sampling (Table 5). To characterize the impact of noise on AUC_{sparse} itself, random noise was also added to the full profiles and 1000 AUCs were generated; the %CV of the 1000 AUCs would reflect the impact of the random noise itself, and any deviation from these %CVs would reflect the effect of the sparse sampling scheme on AUC stability (in the presence of noise). The simulations showed that progressively increasing the noise level resulted in similar AUC means but the CVs increased as expected.

This work was motivated by the need to introduce sparse sampling approaches to reduce the number of animals and the number of plasma samples in toxicokinetic studies. In the area of clinical pharmacokinetics, population-based approaches have been developed to characterize drug kinetics from a few blood samples.[16,17] Some investigators have attempted to reduce the number of animals in pre-clinical (toxicology) studies using compartment-based analysis, within the realm of NONMEM-type population modeling.[18,19] We intentionally avoided this approach because it requires prior knowledge of a drugs' compartmental behavior. If the behavior changes over the course of a toxicology study, and information regarding this is not available, the sparse data may not be amenable to modeling.

In this evaluation, no (statistical) distributions were assumed for the Cp-t data. The re-sampling (bootstrap) technique demonstrated the reliability of the AUCs from sparse sampling, given the variability in the original Cp-t data. Thus, accurate AUC estimates were obtained from sparse sampling (5 points with 2 animals/point) even when the inter-animal variability in Cp-t was very high (40–70%). The scheme was also shown to be applicable when the AUCs for both parent drug and metabolite, viz loratadine and DCL were determined. Since new drug candidates in pre-clinical development generally exhibit PK characteristics and inter-animal variability within the range of those observed for felbamate and loratadine, the scheme with 5 points and 2 rats/point developed in this paper would be applicable to virtually all developmental drugs.

The requirement for implementation of the approach in toxicology studies is information regarding the shape of the Cp-t profiles and the number of "critical" time-points that are needed. Such information can easily be obtained from a single dose study with sampling at multiple time-points. Such a single-dose study will also serve two other goals: (1) assist toxicology in dose selection, and (2) help evaluate PK vs. dose relationship (rough range of linearity). Once a new chemical entity is identified for development, the single-dose PK study would be conducted. Then, the "critical" points can be chosen based on the profile(s), and sampling can be implemented in toxicology studies. The scheme (5 "critical" points with 2 animals/point) amounts to only 10 animals/dose, with 1 blood sample per animal. Thus, for a typical 3 dose level toxicology study in males and females, only 60 blood samples are required. Since only 1 sample is required per animal, it can conveniently be obtained from the core toxicology animals, without the need for separate animals for TK. The same principle is applicable to sparse sampling designs in all species used in safety assessments, and to entire safety programs.

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